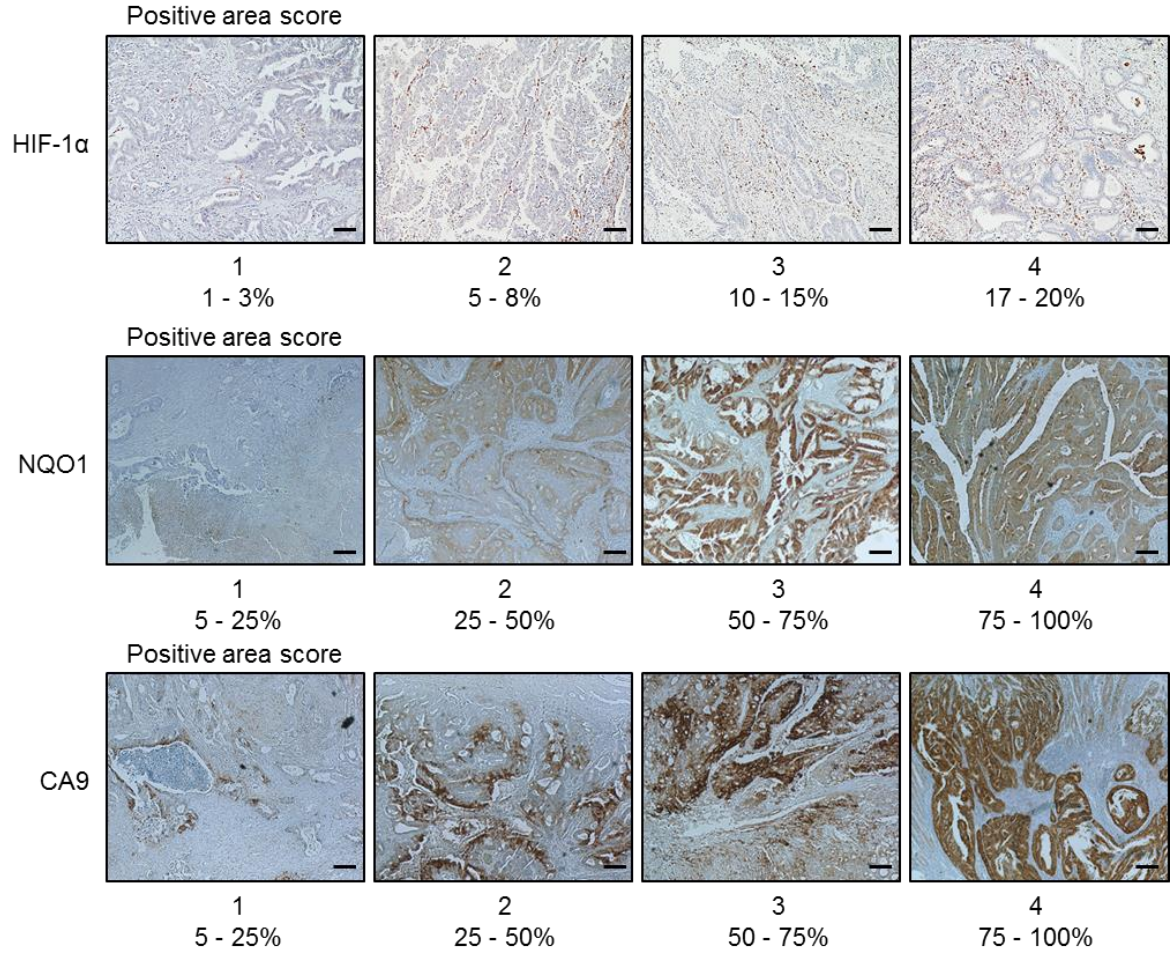
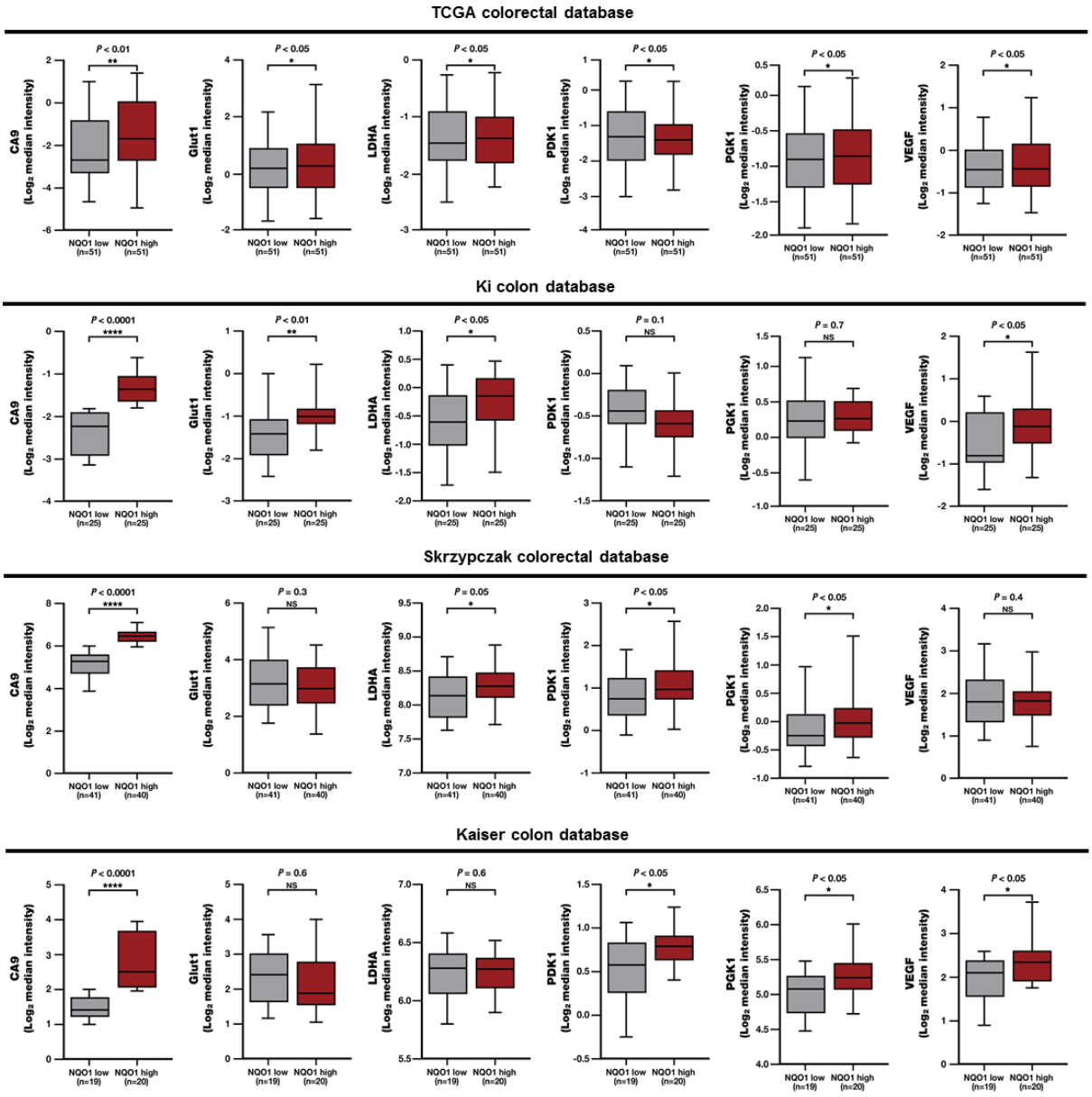
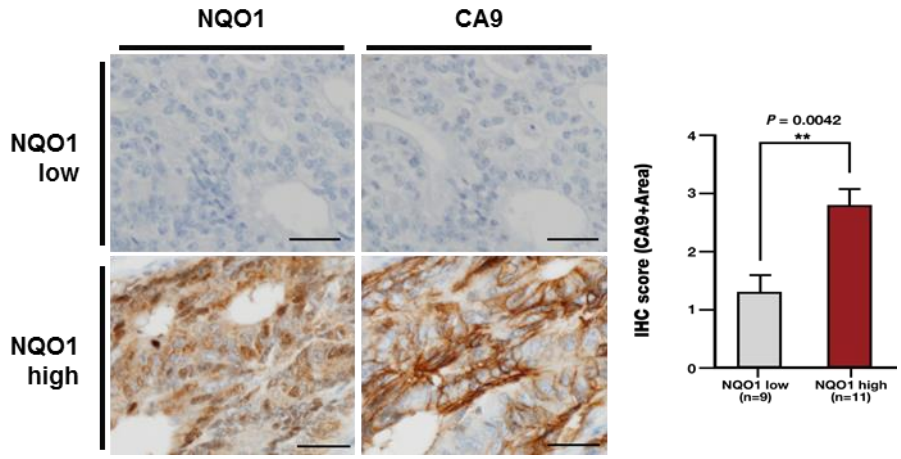


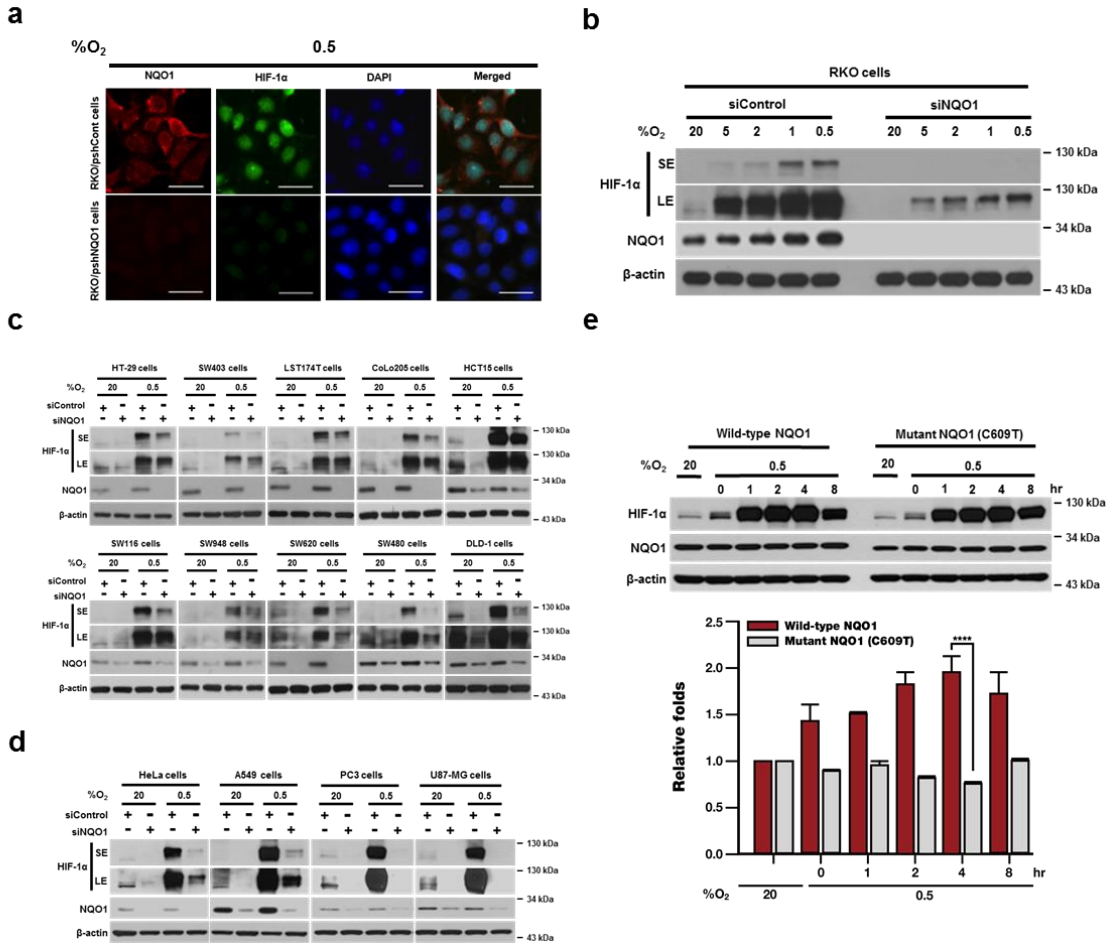
Supplementary Figure 1. Histopathological scoring criteria used for analysis of HIF-1α, NQO1 and CA9 staining in human clinical tumor tissue. HIF-1α IHC score was defined by positive area score (1-3%, 1; >5-8%, 2; >10-15%, 3; >17-20%, 4). IHC scores of NQO1 and CA9 were defined by positive area score (5-25%, 1; >25-50%, 2; >50-75%, 3; >75-100%, 4). Bar = 500 μm



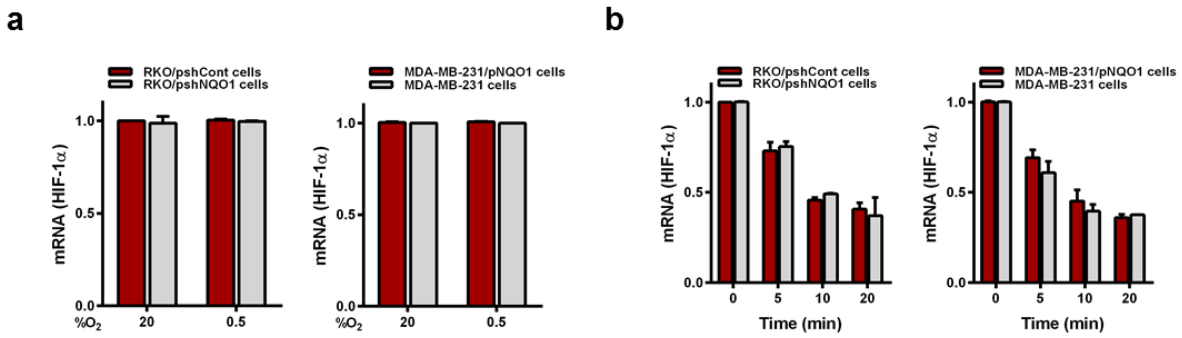
Supplementary Figure 2. Up-regulation of NQO1 correlates with expression of HIF-1α target genes in colorectal cancer patients. Oncomine analysis of the TCGA (n = 51 NQO1 high, n = 51 NQO1 low), Ki (n = 25 NQO1 high, n = 25 NQO1 low), Skrzypczak (n = 40 NQO1 high, n = 41 NQO1 low) and Kaiser (n = 20 NQO1 high, n = 19 NQO1 low) database indicates that the expression of NQO1 correlates with canonical HIF-1α target gene expression. For box plots, the center line represents median value, the box limits are at the 25th and 75th percentiles, and the whiskers represent minimum and maximum values. **P*<0.05 with unpaired *t*-test, ** *P* < 0.01 with unpaired *t*-test, **** *P* < 0.0001 with unpaired *t*-test.



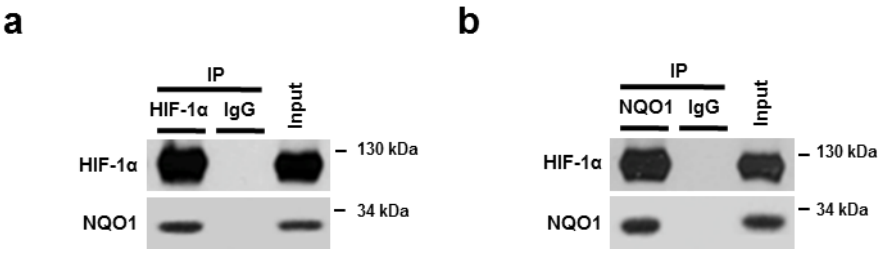
Supplementary Figure 3. Up-regulation of NQO1 correlates with expression of HIF-1α target gene, CA9, in colorectal cancer patients. Correlation between NQO1 and CA9 expression in colorectal cancer tissues. Immunohistochemical detection of CA9 in the high-level expression of NQO1 (n=11) compared to the low-level expression of NQO1 (n=9). Positive area score of CA9 was determined on the most characteristic areas. The positive area score of CA9 was evaluated by determining 10 high magnification power fields (×40). Statistical analysis of the average score of CA9 area score is shown in the right panel (P = 0.0218 with unpaired t-test). Bar = 50 μm



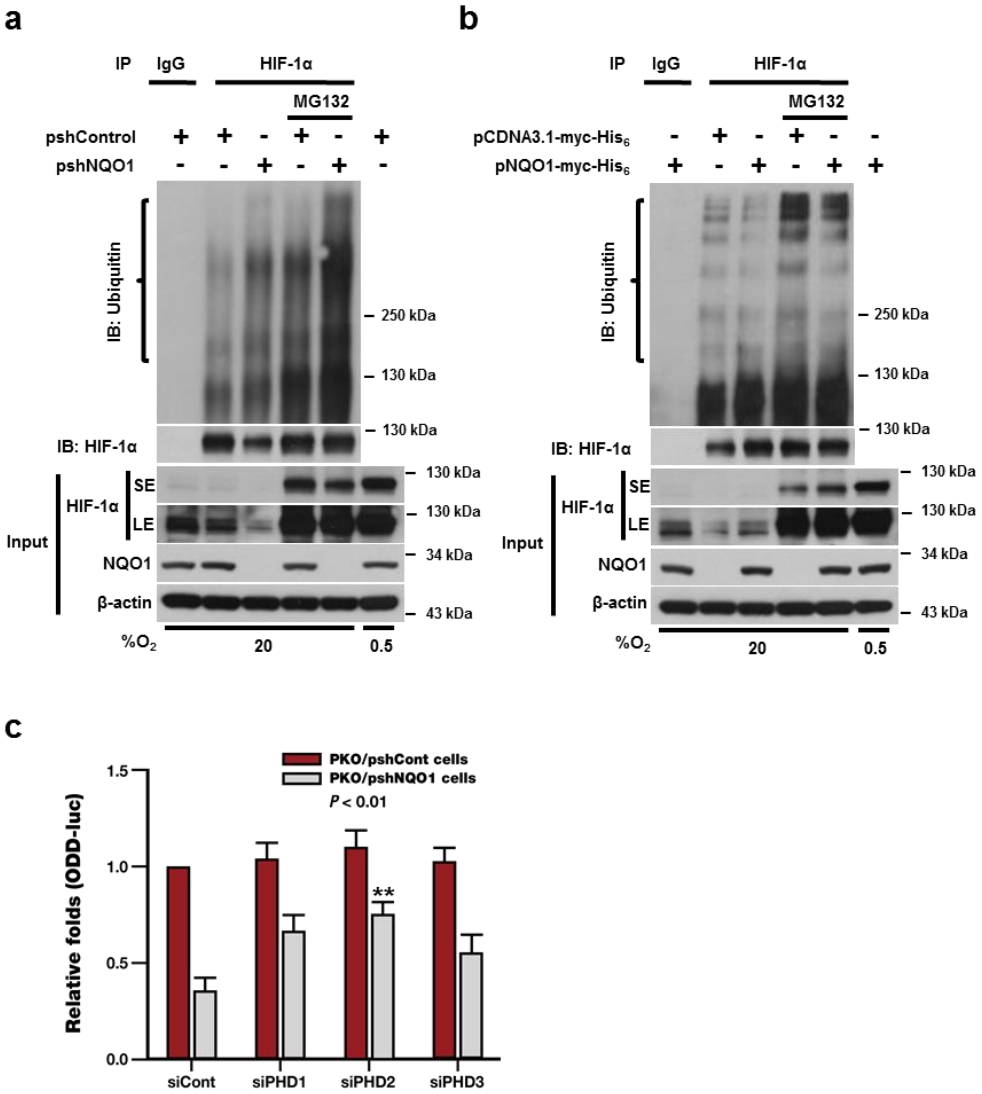
Supplementary Figure 4. Effect of NQO1 on transcription of *HIF-1α* and its stability. (a) NQO1 and HIF-1α expression levels in hypoxic RKO cells (0.5% O_2 for 2 hr) were assessed by immunocytochemical staining with anti-NQO1 primary and FITC-conjugated secondary antibodies, or anti-HIF-1α primary and Texas Red-conjugated secondary antibodies. DAPI was used to stain nuclei. **(b)** Effect of NQO1 on HIF-1α expression levels in various oxygen tensions; 0.5, 1, 2, 5 or 20% O_2. siControl- or siNQO1- transfected RKO cells were exposed to 0.5, 1, 2, 5 or 20% $O_2$for 2 hr and then harvested. Whole-cell lysates were analyzed by immunoblotting for HIF-1α, NQO1 and β-actin. SE, short exposure; LE, long exposure. **(c)** Human colorectal cancer cell lines, including RKO, HT-29, SW403, LST174T, CoLo205, HCT15, SW116, SW948, SW620, SW480, and DLD-1, were transfected with siNQO1. After 48 hr, the cells were exposed to 20% or 0.5% O_2 for 2 hr and then harvested. Whole-cell lysates were analyzed by immnoblotting for HIF-1α, NQO1 and β-actin. SE, short exposure; LE, long exposure. **(d)** A549, PC3, HeLa and U87MG cells were transfected with siNQO1. After 48 hr, the cells were exposed to 20% or 0.5% O_2 for 2 hr and then harvested. Whole-cell lysates were analyzed by immnoblotting for HIF-1α, NQO1 and β-actin. SE, short exposure; LE, long exposure. **(e)** MDA-MB-231/pNQO1 and MDA-MB-231/pNQO1 (C609T) cells were exposed to 20% or 0.5% O_2 for the indicated times. Whole-cell lysates were analyzed by immnoblotting for HIF-1α, NQO1 and β-actin (upper panel), and NQO1 reductase activity (lower panel). The results from three independent experiments are expressed as means ± SEM (P = 0.0001 with ANOVA).



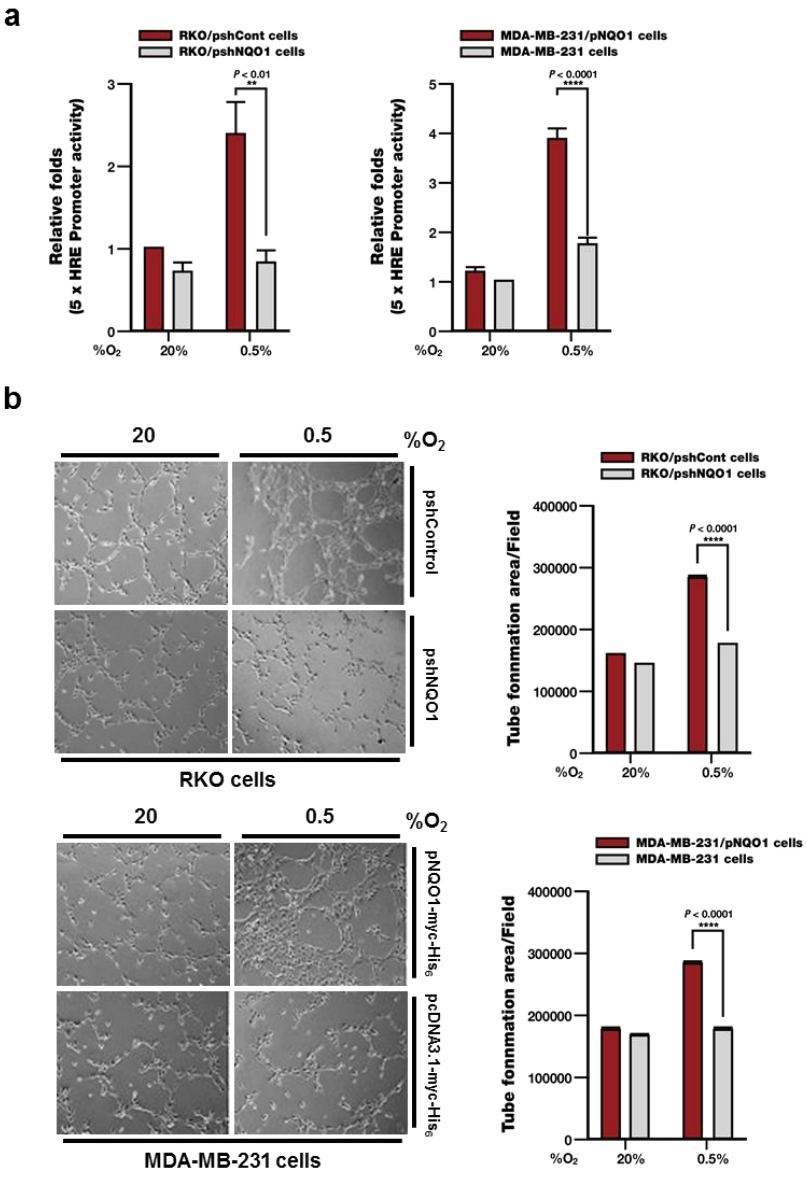
Supplementary Figure 5. NQO1 regulates HIF-1α stability in cancer cells. (a) HIF-1α mRNA expression is not affected by NQO1. RKO cells (Left) and MDA-MB-231 cells (Right) were exposed to 20% and 0.5% O_2 for 2 hr. qPCR was used to detect the expression levels of *HIF-1α*.The mRNA levels of HIF-1α and were examined by qPCR. *18S rRNA* was used as the internal control. **(b)** The effects of NQO1 onHIF-1α mRNA stability. RKO/pshCont and RKO/pshNQO1 cells were exposed to 20% (Left) or 0.5% (Right) O_2 for 2 hr, incubated with 5 μg/ml actinomycin D for 1 hr, and then harvested at the indicated times. The mRNA levels of HIF-1α and were examined by qPCR. *18S rRNA* was used as the internal control.



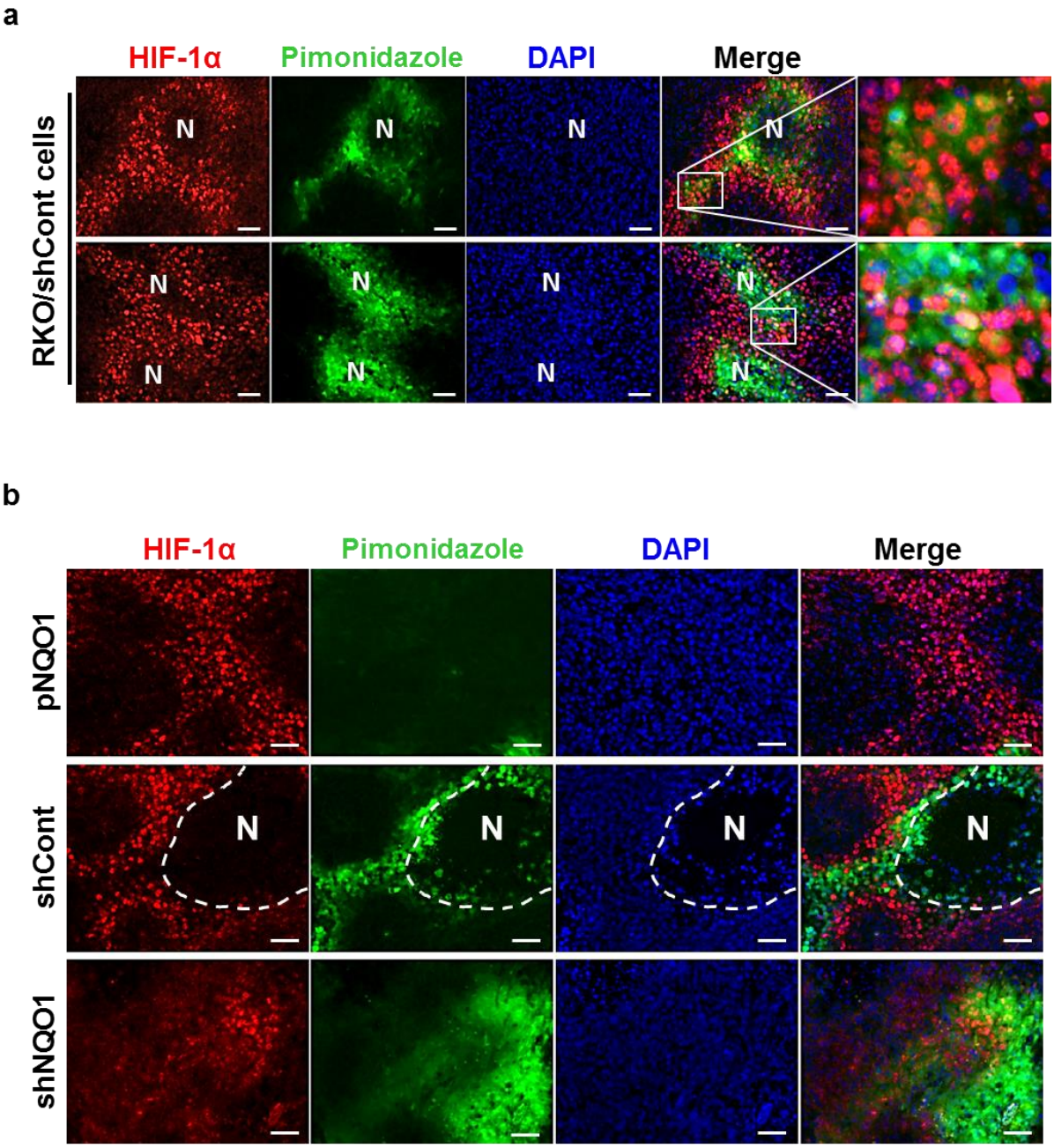
Supplementary Figure 6. NQO1 interacts with HIF-1α in the cytosol. (a,b) MEF cells were exposed to 0.5% O_2. After 2 hr, the cells were harvested and lysates were co-immunoprecipitated with anti-HIF-1α (a) or anti-NQO1 (b) antibody and anti-IgG antibody as a negative control. Then the precipitates were analyzed by an immunoblot analysis with anti -HIF-1α and -NQO1 antibodies.



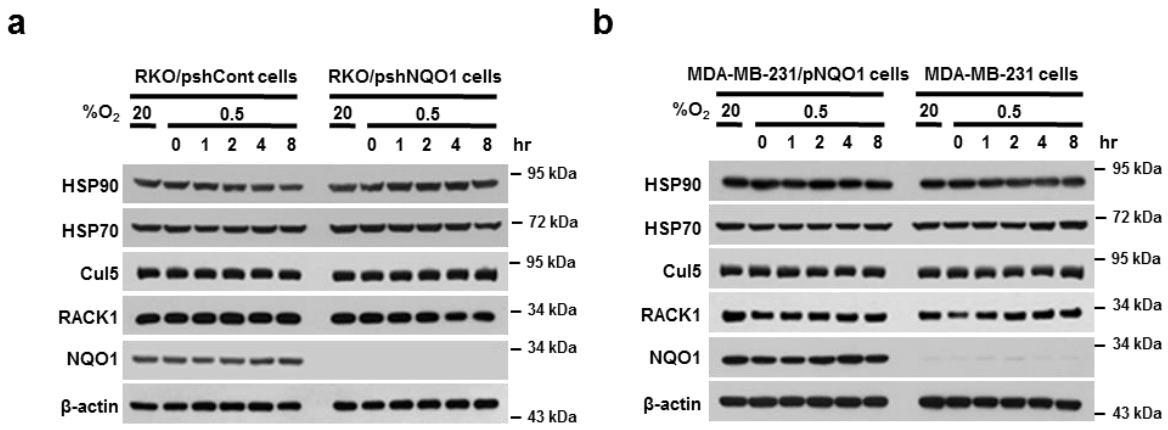
Supplementary Figure 7. NQO1 increases HIF-1α stability by inhibiting ubiquitination and proteasome-mediated degradation. (a,b) RKO (a) and MDA-MB-231 (b) and cells were transfected with pshControl or pshNQO1 and pCDNA3.1-myc-His_6 or pNQO1-myc-His_6 and, respectively. Under non-hypoxic conditions, cells were incubated for 1 hr in the presence or absence of MG132. Whole-cell extracts were immunoprecipitated with an anti- HIF-1α antibody, and ubiquitinated HIF-1α was detected with an anti-ubiquitin antibody. **(c)** RKO/shCont and RKO/pshNQO1 cells were transfected with siPHD1, siPHD2, or siPHD3. After 48 hr, the cells were co-transfected with p*ODD-luc* and pCMV-β-galactosidase for 16 hr, and then exposed to 0.5% O_2 for another 2 hr. Luciferase activity was normalized with respect to β-galactosidase activity (mean ± SD shown; ** $P < 0.01$ with ANOVA).



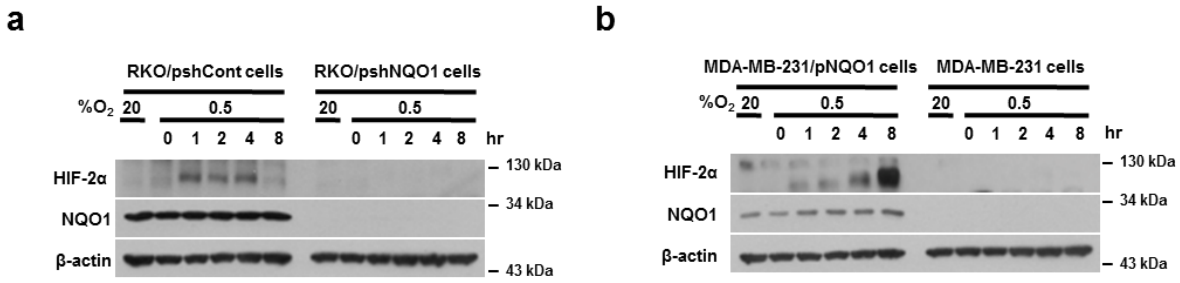
Supplementary Figure 8. NQO1 increases HIF-1α transcriptional activity. (a) RKO (Left) and MDA-MB-231 (Right) cells were transfected with pshCont or pshNQO1 and pCDNA3.1-myc-His_6 or pNQO1-myc-His_6, respectively, for 48 hr, further co-transfected with p5 × HRE-*luc* and pCMV-β-galactosidase for 16 hr, and then exposed to 20% or 0.5% O_2 for another 2 hr. Luciferase activity was normalized with respect to β-galactosidase activity (mean ± SD shown). ** $P < 0.01$ with ANOVA, **** $P < 0.0001$ with ANOVA. **(b)** RKO (upper panel) and MDA-MB-231 (lower panel) cells were exposed to 20% or 0.5% O_2 for 24 hr, and conditioned media were collected. HUVECs (1×10^5 per well) were seeded to 4-well plates in 200 μl of complete ECM plus 800 μl of conditioned media. After 6 hr of incubation, the area covered by the newly formed tubes was determined using the Image J program. The number of pixels in the tube area was quantified for three images per well at 40 × magnification (mean ± SD shown; **** $P < 0.0001$ with ANOVA).



Supplementary Figure 9. NQO1 promotes HIF-1α stabilization. (a) RKO/shCont xenograft tumors were co-stained for pimonidazole (green) and HIF-1α (red). Scale bars = 100 μm. **(b)** HIF-1α expression and tissue hypoxia levels were assessed by immunohistochemical staining in RKO/pNQO1, RKO/pshCont, and RKO/pshNQO1 xenograft tumors. Hypoxic areas in tumors were detected by immunostaining for hypoxia probe, pimonidazole. N, necrotic areas; Scale bars = 100 μm.



Supplementary Figure 10. Effects of NQO1 on expression of proteins related to HIF-1α stability. (a,b) Effects of NQO1 on the expression of proteins related to the degradation of HIF-1α. RKO cells (a) and MDA-MB-231 cells (b) were exposed to 20% and 0.5% O_2 for 8 hr, and cells were harvested at the indicated times. Whole-cell lysates were analyzed by immunoblotting for HSP90, HSP70, Cullin5, RACK1, NQO1, and β-actin.



Supplementary Figure 11. NQO1 enhances HIF-2α expression. (a,b) Effects of NQO1 on the expression of HIF-2α. RKO cells (a) and MDA-MB-231 cells (b) were exposed to 20% and 0.5% O_2 for 8 hr, and cells were harvested at the indicated times. Whole-cell lysates were analyzed by immunoblotting for HIF-2α, NQO1, and β-actin.

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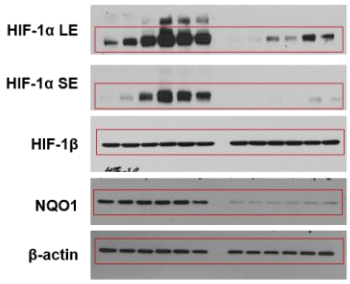

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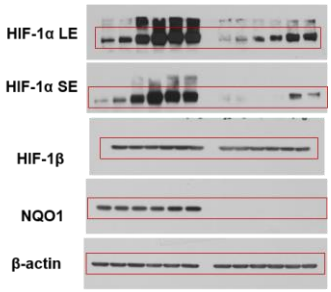

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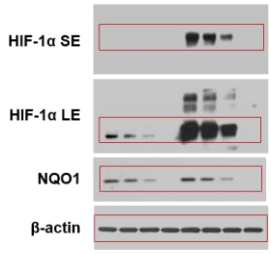

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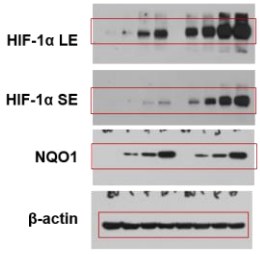

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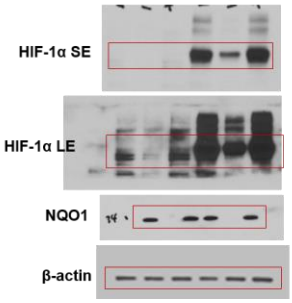

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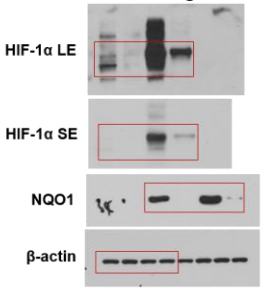

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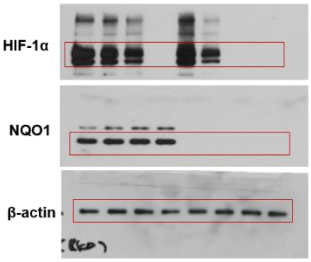

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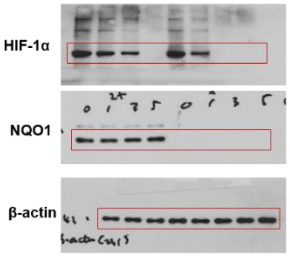

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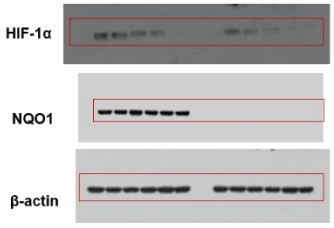

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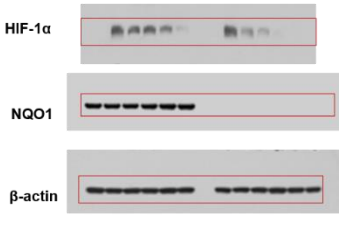

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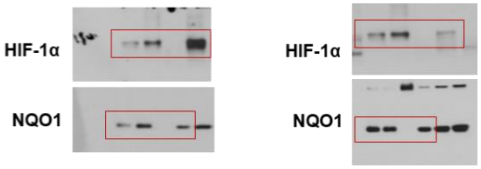

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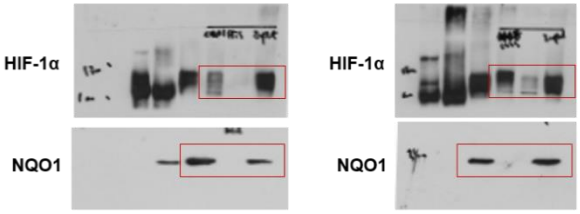

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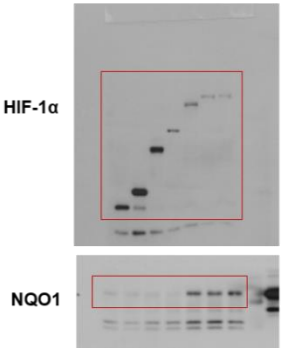

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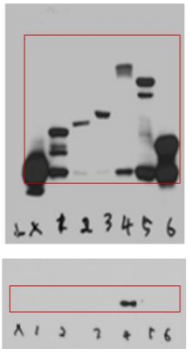

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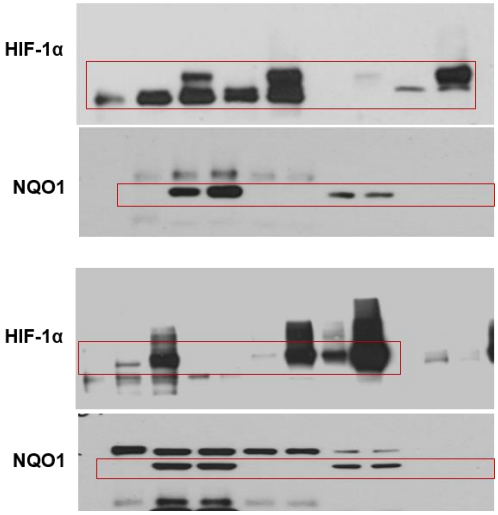

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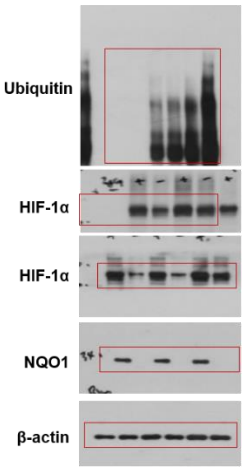

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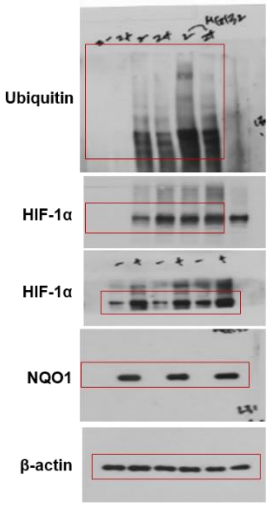

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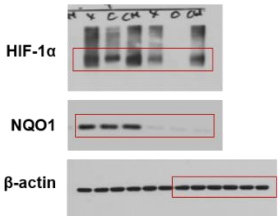

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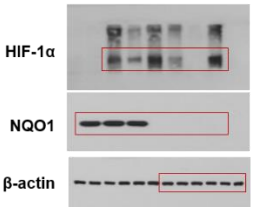

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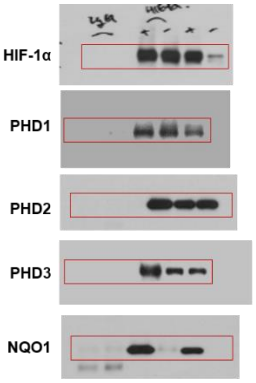

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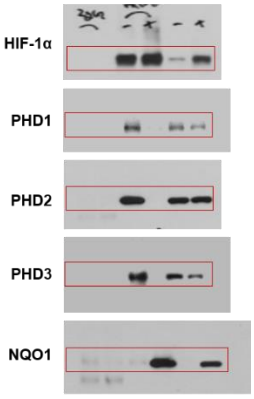

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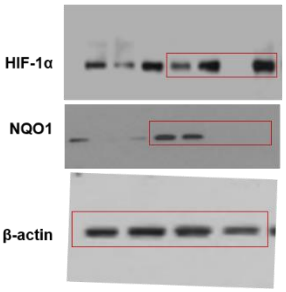

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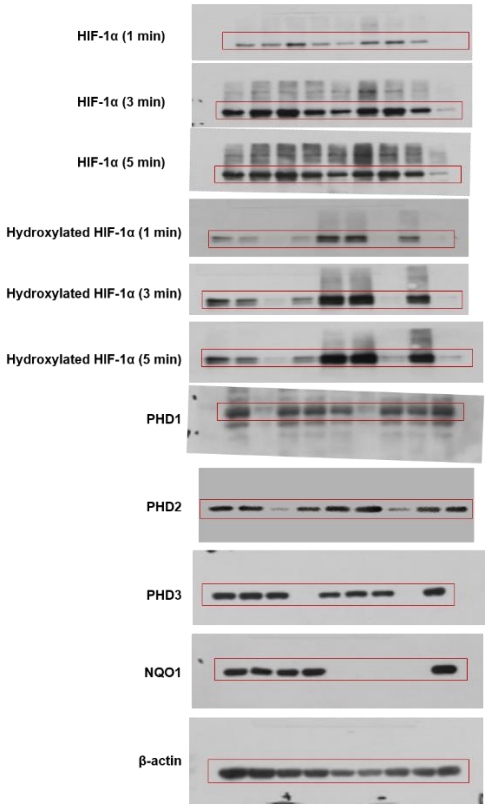

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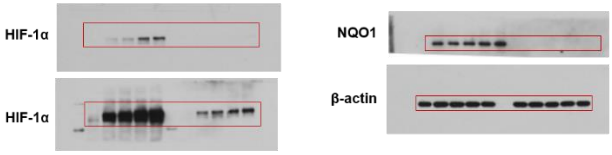

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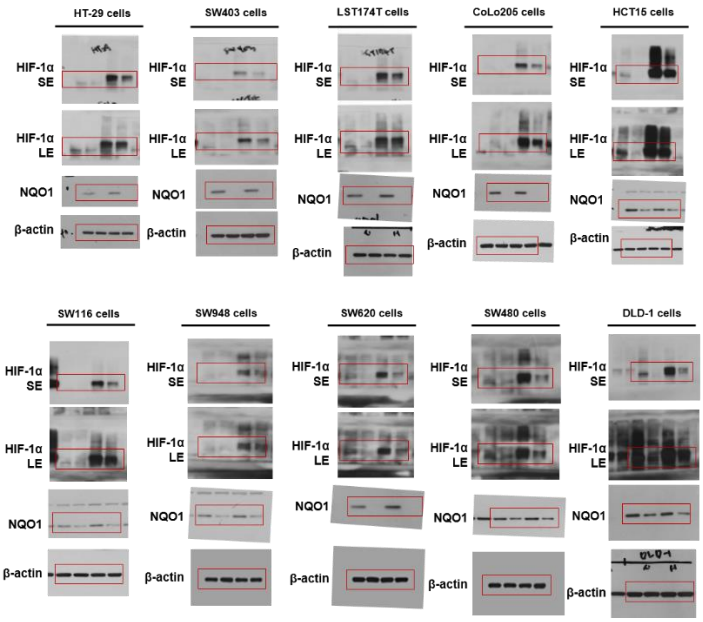

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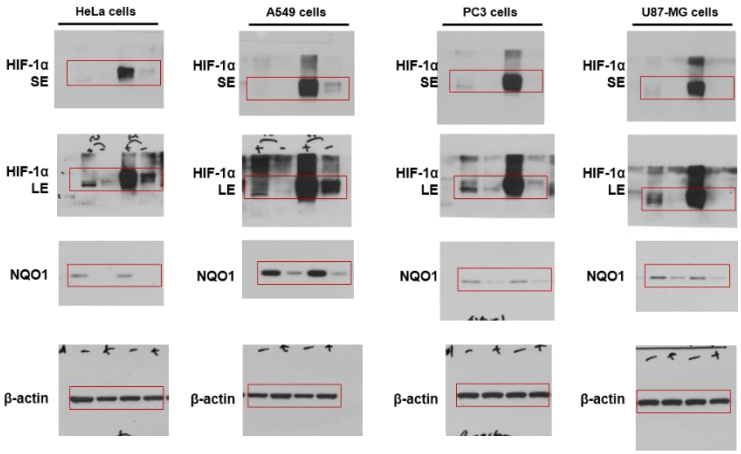

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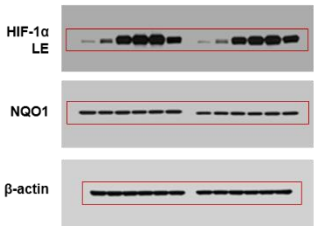

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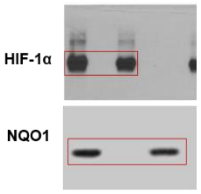

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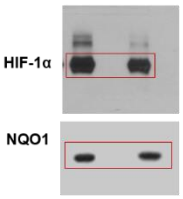

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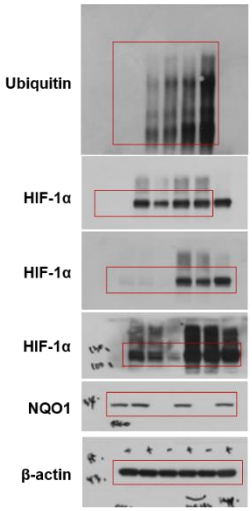

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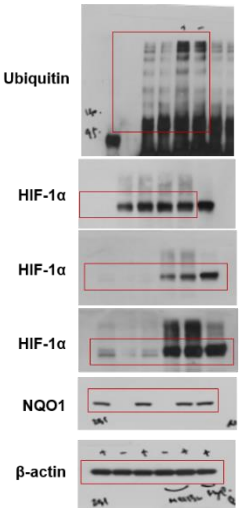

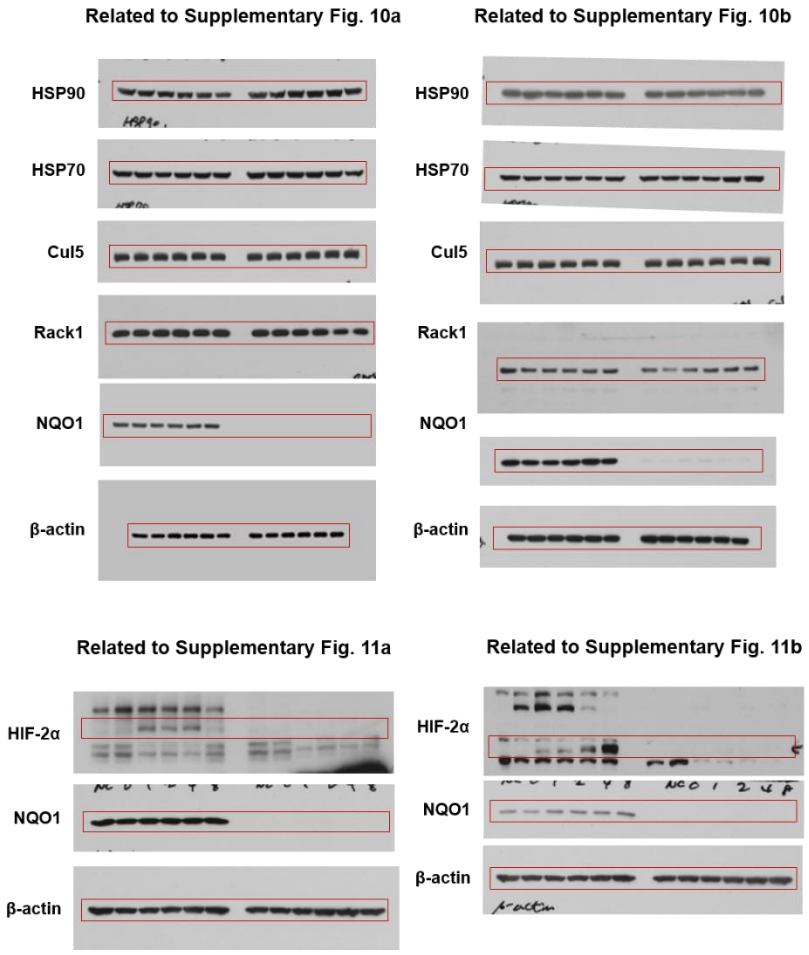

Supplementary Figure 12. Uncropped images of Immunoblots are presented. Reference to the original figures is presented for each blot. (LE = low exposreu; SE = short exposure).